Hereditas 126: 261–268 (1997)

Cytogenetic evaluation of 20 primary breast carcinomas

L. R. CAVALLI[1], L. M. B. CAVALIÉRI[2], L. A. RIBEIRO[3], I. J. CAVALLI[1], R. SILVEIRA[4] and S. R. ROGATTO[3]

[1] *Department of Genetics, Universidade Federal do Paraná, Curitiba, PR*
[2] *Department of Biology, CCB, Universidade Estadual de Londrina, Londrina, PR*
[3] *Department of Genetics, Instituto de Biociências, UNESP, Botucatu, SP, Brazil*
[4] *Oncology Service from the Hospital Nossa Senhora das Graças, Curitiba, PR*

Cavalli, L. R., Cavaliéri, L. M. B., Ribeiro, L. A., Cavalli, I. J., Silveira, R. and Rogatto, S. R. 1997. Cytogenetic evaluation of 20 primary breast carcinomas. — *Hereditas 126*: 261–268. Lund, Sweden. ISSN 0018-0661. Received March 24, 1997. Accepted June 14, 1997

Chromosome analysis was performed on samples from 20 Brazilian patients with breast cancer. All the samples were from untreated patients who presented the clinical symptoms for months or years before surgical intervention. Six cases showed axillary lymph node metastases. Clonal chromosome abnormalities were detected in all cases. The numerical alterations most frequently observed involved the loss of chromosomes X, 19, 20, and 22 followed by gain of chromosomes 9 and 8. Among the structural anomalies observed, there was preferential involvement of chromosomes 11, 6, 1, 7, 3, and 12, supporting previous reports that these chromosomes may harbour genes of importance in the development of breast tumors. Two cases with a family history of breast cancer had in common total or partial trisomy 1.

Silvia Regina Rogatto, Departamento de Genética, IB UNESP—Botucatu—São Paulo CEP 18607-070-Brazil

Breast cancer is the most common neoplasm and the leading cause of cancer related deaths among women in most countries (PARKIN et al. 1984; BORING et al. 1992). According to the American Cancer Society (1994), the incidence of breast cancer has risen steadily with a cumulative risk of 12.6%, or one in eight women by age 85 for a girl born in 1990.

The natural history of breast cancer varies greatly according to age at onset, clinical features, histopathological characteristics, and genetic context. Although the majority of cases appear to be sporadic, a family history of breast cancer is considered to be the main risk factor. *BRCA1* located on chromosome band 17q21 (MIKI et al. 1994), and *BRCA2* mapped on 13q12–13 (WOOSTER et al. 1994) are considered to be responsible for most cases of hereditary breast cancer. Somatic mutations involving these genes in sporadic breast cancer are rare (FORD et al. 1994; FUTREAL et al. 1994; MIRAJVER et al. 1995). However, recent reports have suggested role for *BRCA1* in nonhereditary breast cancer (FITZGERALD et al. 1996; LANGSTON et al. 1996; VAUGHN et al. 1996).

One potential marker for the tumorigenesis process is the degree of genetic change in the tissue at risk. Cytogenetic investigation using chromosome banding is a screening methodology that permits the analysis of individual cells. This method permits the assessment of intratumoral heterogeneity (PANDIS et al. 1994, 1996) as well as the identification of specific breakpoints that may indicate the existence of other as yet unidentified breast cancer genes.

Although there are many reports on the cytogenetics of breast cancer, data about chromosome alterations in primary tumors are limited, and there is very little information on clinical-histopathological features correlated with specific chromosomal alterations (HAINSWORTH et al. 1992; ZAFRANI et al. 1992; PANDIS et al. 1993, 1996; TRENT et al. 1993; STEINARSDÓTTIR et al. 1995).

The objective of the present study was to investigate the karyotypic patterns of twenty breast carcinomas from Brazilian patients to obtain information on the spectrum of chromosomal alterations and, when possible, to correlate the results with clinical-histopathological characteristics and information on family history.

MATERIALS AND METHODS

Twenty untreated primary breast carcinomas (15 infiltrating ductal, 1 infiltrating lobular, 1 infiltrating tubular, and 3 intraductal carcinomas) were evaluated cytogenetically after short-to-medium term in vitro culture. The tumor samples obtained from 19 female patients and 1 male patient were collected from the Hospital Nossa Senhora das Graças de Curitiba, Curitiba, and from the Hospital Universitário Regional Norte do Paraná, Londrina, state of Paraná, South Brazil. The tumors were classified morphologically according to the recommendations of the World Health Organization (WHO) (SOBIN 1981).

Table 1 summarizes the clinicopathologic findings for the 20 breast carcinomas. All cases were classified as large size since they were more than 20 mm in diameter. Six patients showed axillary lymph node metastases.

Table 1. *Clinical and histopathological data of the primary breast carcinomas analyzed*

Case	Histopathological diagnostic	Age (years)/ sex	Family/personal history	Lymph node involvement
01	Infiltrating ductal carcinoma	48/F	–	–
02	Infiltrating ductal carcinoma	57/F	–	nd
03	Infiltrating ductal carcinoma	61/F	Lung cancer (brother)	nd
04	Infiltrating ductal carcinoma	42/F	Fibroadenoma in the opposite breast;	–
			Breast cancer (two sisters and two paternal aunts)	–
05	Infiltrating ductal carcinoma	36/F	Lung cancer (father and brother)	–
06	Infiltrating ductal carcinoma	49/F	–	–
07	Infiltrating ductal carcinoma*	69/M	–	+
08	Infiltrating ductal carcinoma	41/F	nd	+
09	Infiltrating ductal carcinoma	42/F	nd	–
10	Infiltrating ductal carcinoma	73/F	–	+
11	Infiltrating ductal carcinoma	39/F	–	+
12	Infiltrating ductal carcinoma	57/F	nd	nd
13	Infiltrating ductal carcinoma	56/F	–	–
14	Infiltrating ductal carcinoma	48/F	–	+
15	Infiltrating ductal carcinoma	68/F	–	–
16	Intraductal carcinoma	62/F	–	–
17	Intraductal carcinoma	81/F	–	–
18	Intraductal carcinoma	75/F	–	–
19	Infiltrating tubular carcinoma	45/F	–	–
20	Infiltrating lobular carcinoma	43/F	Breast cancer (sister)	+

(F): Female (M): Male (nd): Not determined
(+):Present (–): Absent
* Previously published

A fragment of tumor close to that taken for histologic examination was brought to the cytogenetics laboratory. The tumor tissue fragments for chromosome evaluation were sectioned after removal of fatty and necrotic areas. Chromosome preparation was performed according to a previously described protocol (CAVALLI et al. 1995). Chromosome studies were conducted only on primary cultures. G-banding (SCHERES 1972) and cytogenetic analysis followed routine methods. A total of 7-33 metaphases were fully karyotyped, according to the number of mitoses obtained in each case. Chromosomes were identified and classified according to the nomenclature proposed by the International System for Human Cytogenetic Nomenclature (ISCN 1995).

RESULTS

Cellular morphology showed growth of epithelial cells in early primary cultures. Metaphases were obtained from dividing epithelial cells in the early growth cycle to prevent the overgrowth of fibroblasts observed in older cultures. On average, 15-day old cell cultures with high mitotic activity of epithelial cells were prepared for cytogenetic investigation.

Clonal karyotypic alterations were observed in all cases analyzed. Numerical changes were more frequently observed, involving the loss of chromosomes X (10 cases), 19 (7 cases), 20 and 22 (5 cases), followed by the gain of chromosomes 9 (5 cases) and 8 (4 cases). Structural chromosome anomalies preferentially involved chromosomes 11 (6 cases), 6 (5 cases), 1 (4 cases), and 7 (3 cases) (Fig. 1 and 2).

The presence of chromosome markers was detected in 40 % of the tumors. Nonclonal changes (not shown) were detected in all specimens; some of them identical to the clonal abnormalities seen in other cases [e.g., del(6q), +8 and del(11p)]. Normal cells were detected in 19 cases at frequencies ranging from 12 to 66 %.

The number of cells after G-banding analysis and the composite karyotype observed in each case are presented in Table 2.

DISCUSSION

The breast tumor is structurally composed by epithelial cells, stroma elements and other cell types (PONTÉN et al. 1990). Interactions among these cellular types are essential for successful tumoral growth, although the nature of these intratumor cell interactions are still unknown. One major limitation in in vitro breast tumor research is that myoepithelial cells have a doubling time of 24 hours and normal epithelial cells have a doubling time of 48 hours, while the population of cancer cells may take one week or

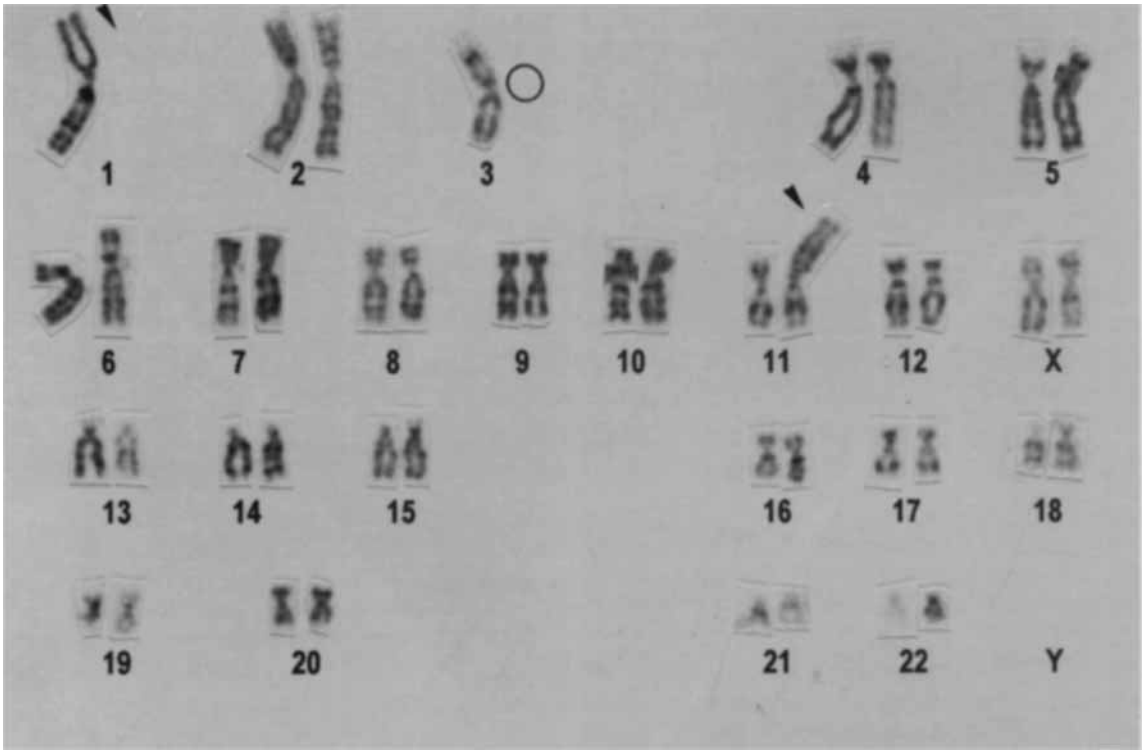

Fig. 1. Cell with a 45,XX,t(1;11)(p13;p11.2),−3 karyotype (case 14 — Infiltrating ductal carcinoma).

more to double (PETERSON and VAN DEURS 1987). Thus, separation of tumor cells from nonmalignant epithelium after in vitro culture is still a problem. On this basis, the presence of karyotypically normal cells is expected and frequently observed in breast tumor cultures.

Another important aspect is whether the medium used or the time of growth in culture favors the overgrowth of specific near-diploid cell lines. Different tissue culture techniques using specific medium for mammary tumor cells have been recently developed (PANDIS et al. 1992; STEINARSDÓTTIR et al. 1995). Differences were detected in the karyotypic profile according to the cell culture method used. However, the chromosomes more frequently involved regardless of the methods used, were chromosomes 1, 6, 8, 11, 3, 7, 16, 17, and 18, which are reported in the majority of cytogenetic studies on breast tumors (TRENT 1985; HILL et al. 1987; DUTRILLAUX et al. 1990; HAINSWORTH and GARSON 1990; MITCHELL and SANTIBANEZ-KOREF 1990; BULLERDIEK et al. 1993; LU et al. 1993; PANDIS et al. 1993, 1995; THOMPSON et al. 1993; TRENT et al. 1993; BIÈCHE et al. 1995; ROHEN et al. 1995; STEINARSDÓTTIR et al. 1995). In the present study, we found the nonrandom involvement of the same chromosomes as described above in addition to abnormalities involving chromosomes X, 19, 20, and 22. The chromosome abnormalities observed are in agreement with the results reported in the literature. However, we cannot rule out the possibility that the time in culture and/or the method used favored the preferential growth of these cells in vitro.

Most authors consider that chromosome 1 is the most frequently altered in breast cancer, often showing monosomy 1 or 1p, polysomies for 1q and other rearrangements including deletions, amplifications and translocations on the two chromsome arms (BIÈCHE and LIDEREAU 1995). In our study, we found total or partial trisomy 1 and deletion and translocation involving chromosome 1. All these patients had a histopathological diagnosis of infiltrating carcinoma, three of them ductal, one lobular and one tubular. Patient age was 41–43 years. In two of these cases there was a family/personal history of breast cancer (cases 4 and 20), and lymph node metastases were observed in one case (case 20). These clinical-histopathological features and the presence of abnormalities of chromosome 1 may be associated with tumor progression. HAINSWORTH et al. (1992) found a significant correlation between rearrangements on chromosome 1 and poor prognosis. Losses of short arm of chromosome 1 are interpreted as secondary anomalies acquired during clonal evolution (PANDIS et al. 1995).

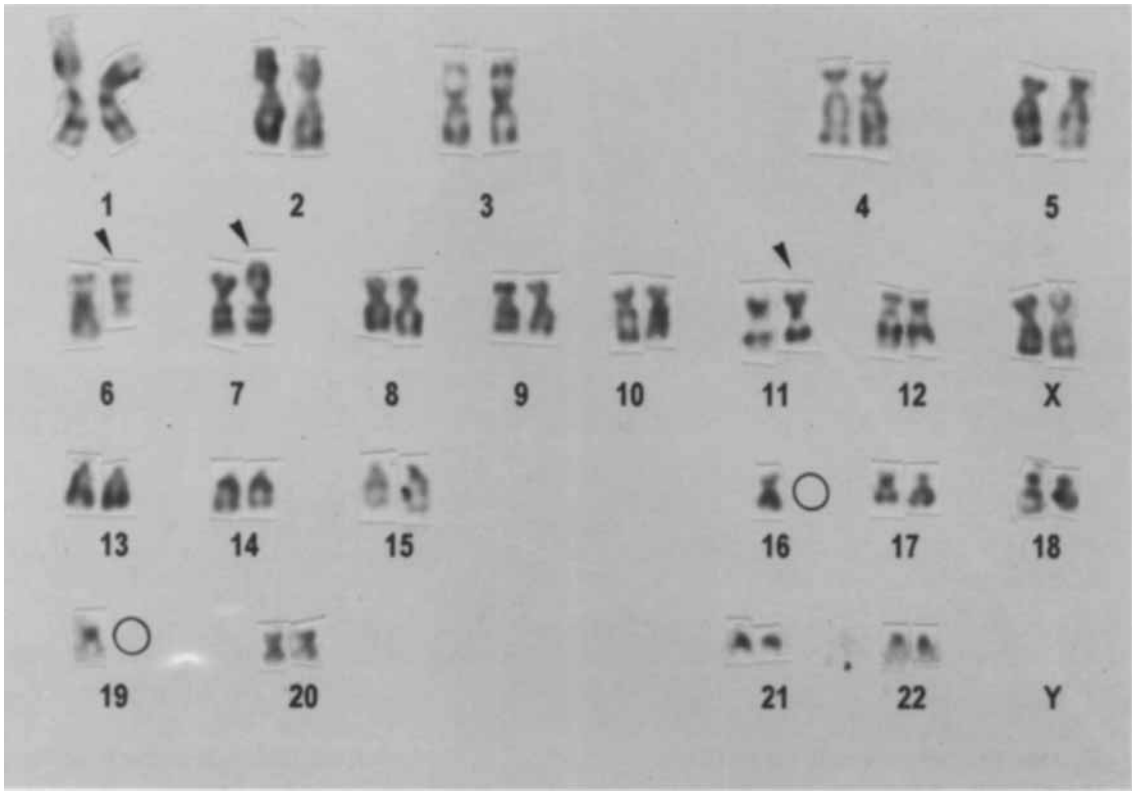

Fig. 2. Representative G-banded karyotype of the case 2 (infiltrating ductal carcinoma). Arrowheads indicate del(6)(q15), add(7)(p22) and del(11)(q23). Monosomy 16 and 19 are non-clonal.

Deletions on 6q were observed in four cases of infiltrating carcinomas (three of them ductal and one tubular) and in one case of intraductal carcinoma (case 17). This abnormality is frequently described in breast carcinomas (DUTRILLAUX et al. 1990; HAINSWORTH et al. 1991; LU et al. 1993; PANDIS et al. 1993; THOMPSON et al. 1993). TRENT et al. (1993) observed deletions in 6q in the analysis of 34 metastatic breast tumors and provided evidence for the first model of progression of karyotypic alterations from primary localized to metastatic breast carcinoma. According to this model, the first step towards the progression may be associated with the alteration of one or more genes that may be located on one of the chromosomes frequently altered in breast cancer (e.g., 1p or 6q), which in turn may lead to generalized chromosome instability. Recently, some reports on benign proliferative breast diseases revealed the presence of deletions involving 6q (FLETCHER et al. 1991; LEUSCHNER et al. 1994; DAL CIN et al. 1996). Deletions in 6q in benign tumors and breast carcinomas suggest that this abnormality may affect genes involved in the initial stages of mammary tumorigenesis. Alternatively, at the molecular level, the region 6q21-25 can harbor more than one gene that may possibly contribute to different steps in human tumorigenesis considering the high frequency and breakpoint heterogeneity of this deletion in the different tumors (ref. in MITELMAN 1994). Molecular studies of breast carcinomas (DEVILEE et al. 1992; NEGRINI et al. 1994; FUJII et al. 1996) and of other tumors (MILLIKIN et al. 1991; FOULKES et al. 1993) revealed the loss of heterozygosity in 6q. The findings of 6q deletions after cytogenetic and molecular analysis suggest that one or more putative tumor suppressor genes might exist at this site.

Trisomy of chromosome 8, detected in 4/15 invasive ductal carcinomas, has been found by other authors in studies on the same type of pathology (BULLERDIEK et al. 1993; BIÈCHE et al. 1995; ROHEN et al. 1995). BULLERDIEK et al. (1993) reported trisomy 8 in 2 of 16 cases which presented lymph node involvement and were positive for estrogen and progesterone receptors. In our study, two of four cases with trisomy 8 presented lymph node involvement. In general, 8p is known to be a common site of homogeneously staining regions in breast tumors (DUTRILLAUX et al. 1990). At present, the pathogenetically relevant locus involved in these amplifications is unknown. Comparison of the clinical-

Table 2. *Number of cells after G-banding and composite karyotype observed in the 20 primary breast carcinomas*

Case	No. of cells/ G-banding	Composite karyotype
01	19	44~47,X,−X,del(11)(p15),del(11)(q23),+13,−22[cp16]/46,XX[3]
02	22	43~48,XX,del(6)(q15),add(7)(p22),del(11)(q23)[cp22]
03	33	45~48,XX,del(6)(q23),+del(12)(q22),+mar1,+mar2[cp21]/46,XX[12]
04	08	45~55,XX,+1,−16[cp5]/46,XX[3]
05	17	44~45,X,−X,−7,+mar[cp6]/46,XX[11]
06	16	43~48,XX,+8,+9,del(12)(q21q24.1)[cp13]/46,XX[3]
07	25	44~48,XY,+8,+9,−17,add(17)(q25),+mar[cp22]/46,XY[3]
08	27	40~47,X,−X,add(X)(q28),+add(1)(p10),−4,+8,+9,del(11)(q12q14),−17,−19,+mar1, +mar2[cp24]/46,XX[3]
09	15	40~46,XX,del(7)(q32),del(11)(q12q14),−19,−22[cp12]/46,XX[3]
10	28	42~48,X,−X,−3,t(3;6)(q11;p25),del(7)(p12p14),+14,−18,−19,−20,+mar[cp19]/46,XX[9]
11	15	43~45,XX,−19[cp10]/46,XX[5]
12	25	42~48,X,−X+7,+9,+10,−11,−14,+14,−15,−17,+18,−19,−20,+21,+mar[cp22]/46,XX[3]
13	16	43~48,X,−X,+7+8,+13,+20,−21,+mar[cp14]/46,XX[2]
14	23	42~46,X,−X,t(1;11)(p13;p11.2),−3−15,−20,−22[cp22]/46,XX[1]
15	07	45,X,−X[6]/46,XX[1]
16	27	43~47,X,−X,−19,+mar1,+mar2[cp16]/46,XX[11]
17	25	43~47,XX,del(4)(p14),del(6)(q24),+7,del(18)(p11.2),−22[cp17]/46,XX[8]
18	27	39~49,C,−X,inv(3)(p11p25),−7,add(11)(p15),+18,−19,−20,−21[cp22]/46,XX[5]
19	19	42~47,XX,del(1)(q42),del(6)(q24),add(13)(q34),−20,−22[cp13]/46,XX[6]
20	11	44~48,XX,+add(1)(p10),+9,+22[cp6]/46,XX[5]

histopathological data for patients with or without trisomy 8 may confirm the role of this alteration in the etiology of infiltrating carcinomas.

Total or partial loss involving chromosome 11 is frequently described in breast tumors, with regions 11p15 and 11q23-24 being commonly involved (ref. MITELMAN 1994). In our study, we found del(11)(p15) (case 1), and del(11)(q23) (cases 1 and 2), del(11)(q12q14) (cases 8 and 9), add (11)(p15) (case 18), and monosomy 11 (case 12). FERTI-PASSANTONOPOULOU et al. (1991) detected deletions at these sites in 20/30 cases of breast carcinomas and suggested that these abnormalities were associated with tumoral progression and were indicative of poor prognosis. Chromosome 11 has been shown to be the site of oncogenes *H-RAS*, *ETS*, and *INT*, mapped to 11p15, 11q13 and 11q23, respectively; of the *WT1* gene on 11p, of the *MEN1* locus on 11q13, and of the ataxia telangiectasia gene mapped to the 11q22-23 region. Loss of heterozygosity (LOH) on 11p has been correlated with low estrogen receptor protein and tumor size in invasive breast carcinoma, both of which are indicators of a poor prognosis (MACKAY et al. 1988). LOH for 11q23 either alone or in conjunction with LOH for 11p15.5 in breast tumors was found to be highly predictive of an aggressive post-metastatic disease course with reduced survival (WINQVIST et al. 1995). GUDMUNDSSON et al. (1995) investigated LOH on chromosome 11 and found an association with positive node status in the 11p15.5 and 11p13 regions, and LOH at 11p15.5 and 11q22-qter region was correlated with a high S-phase fraction. If these observations hold true in other cases, screening for deletions in 11p15 or 11q23 may be useful for identifying patients at high risk to develop aggressive disease.

Structural alterations involving chromosome 7 was found in three cases (cases 2, 9, and 10). The breakpoint regions involved in the deletions of this chromosome are coincident with the localization of the oncogene *EGFR* (mapped on 7p11-13) and the *MET* oncogene (mapped on 7q31). BIÈCHE et al. (1992) showed that patients whose breast cancer carried a deletion on chromosome band 7q31 had a significantly higher risk of relapse and of death, and had a shorter metastasis-free and overall survival than those whose tumors did not have such a deletion. It is interesting to note that case 2 presented structural alterations in chromosome 11 and 7, both changes being related to a poor prognosis. Trisomy 7 was observed in three other cases (cases 5, 12, and 17). A similar trisomy has been detected in other series of breast tumors and is accepted as a primary chromosome anomaly (PANDIS et al. 1995).

Loss of chromosomes X, 19, 20, and 22 was found in all the histological types of breast tumors analyzed (except in the lobular type) in our study. Loss of chromosome X was the most frequent alteration detected in our cases. This abnormality and some others (e.g., +7, +10) have been reported in various tumors, but it has been questioned whether they were derived from neoplastic cells (LINDSTRÖM et al. 1991;

JOHANSSON et al. 1993; ROGATTO et al. 1994). Recently, analysis of loss of heterozygosity and deletion mapping on the X chromosome in human breast tumors revealed a high frequency of small regionalised deletions defining at least three regions independent: (a) distal portion of a pseudoautosomal region of Xp; (b) within the pseudoautosomal region close to the pseudoautosomal boundary; and (c) distal Xq (LOUPART et al. 1995). This study suggested the presence of suppressor genes involved in breast cancer at these sites.

Monosomies of chromosome 19 were described in metastatic breast tumors after short-term culture (7–22 days) (TRENT et al. 1993). Loss of chromosome 19 was not found in direct chromosome analysis of primary breast carcinomas (HAINSWORTH et al. 1991; LU et al. 1993). However, HAINSWORTH et al. (1991) and PANDIS et al. (1995) detected rearrangements involving 19q13 in breast carcinomas. Homogeneously staining region (hsr) on 19q was the most common site of hsr in primary breast tumors analyzed after short-term culture (24–48 hours) (DUTRILLAUX et al. 1990). These observations demonstrated that the chromosomal pattern detected depends on the techniques used (PANDIS et al. 1994), and therefore it is possible that the primary event was hsr on chromosome 19. Loss of chromosome 19 detected cytologically could be the result of the time of culture in vitro as well as the instability of hsr itself on 19q.

Finally, although chromosomal heterogeneity occurs in breast carcinomas, we observed in our samples the same alterations previously described by other authors. Cytogenetic analysis of breast tumors continues to be an important tool for the understanding of cancer biology by the delineation of specific regions for further molecular studies. Additionally, certain genetic abnormalities appear to be of prognostic value in oncology and should be further investigated.

ACKNOWLEDGMENTS

The authors thank the staff of the Oncology Service from Hospital Nossa Senhora das Graças, Curitiba; Hospital Universitário Regional do Norte do Paraná, Londrina, Paraná, Brazil. The present study was supported by grants from CNPq (National Research Council for Scientific and Technologic Development).

REFERENCES

American Cancer Society, (1994). Cancer Facts and Figures. American Cancer Society, Atlanta.

Bièche I and Lidereau R, (1995). Genetic alterations in breast cancer. Genes Chromosom. Cancer 14: 227–251.

Bièche I, Champeme MH, Matifas F, Hacène K, Callahan R and Lidereau R, (1992). Loss of heterozygosity on chromosome 7q and aggressive primary breast cancer. Lancet 339: 139–143.

Bièche I, Meyer-Bolte K, Bonk U, Ebel T, Staats B, Leuschner E, Gohla G, Caselitz J, Bartnitzke S and Bullerdiek J, (1995). Trisomy 8 and 18 as frequent clonal and single cell aberrations in 185 primary breast carcinomas. Cancer Genet. Cytogenet. 80: 33–39.

Boring CC, Squires TS and Tong T, (1992). Cancer statistics. CA Cancer J. Clin. 41: 19–36.

Bullerdiek J, Leuschner E, Taquia E, Bonk U and Bartnitzke S, (1993). Trisomy 8 as a recurrent clonal abnormality in breast cancer. Cancer Genet. Cytogenet. 65: 64–67.

Cavalli LR, Rogatto SR, Rainho CA, Santos MJ, Cavalli IJ and Grimaldi DM, (1995). Cytogenetic report of a male breast cancer. Cancer Genet. Cytogenet. 81: 66–71.

Dal Cin P, Moerman P, Christiaens MR and Van Den Berghe H, (1996). Observation of a deletion of the long arm of chromosome 6 in benign fibrocystic disease of the breast constitutes a challenging problem. Genes Chromosom. Cancer 16: 68–71.

Devilee P, Van Vliet M, Van Sloun P, Dijkshoorn NK, Hermans J, Pearson PL and Cornelisse CJ, (1992). Allelotype human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. Oncogene 6: 1705–1711.

Dutrillaux B, Gerbault-Seureau M and Zafrani B, (1990). Characterization of chromosomal anomalies in human breast cancer. A comparison of 30 paradiploid cases with few chromosome changes. Cancer Genet. Cytogenet. 49: 203–217.

Ferti-Passantonopoulou A, Panani AD and Raptis S, (1991). Preferential involvement of 11q23-24 and 11p15 in breast cancer. Cancer Genet. Cytogenet. 51: 183–188.

Fitzgerald MG, MacDonald DJ, Krainer M, Hoover J, O'Neil E, Unsal H, Silva-Arrieto S, Finkelstein DM, Beer-Romero F, Englert C, Sgroi DC, Smith B, Younger JW, Garber JE, Duda RB, Mayzel KA, Isselbacher KJ, Friend SH and Haber DA, (1996). Germline BRCA1 mutations in Jewish and non-Jewish women with early-onset breast cancer. N. Engl. J. Med. 334: 143–149.

Fletcher JA, Pinkus GS, Weidner N and Morton CC, (1991). Lineage restricted clonality in biphasic solid tumors. Am. J. Pathol. 138: 1199–1207.

Ford D, Easton D, Bishop D, Narod S and Goldgar D, (1994). Risks of cancer in BRCA-1 mutation carriers. Lancet 343: 692–695.

Foulkes WD, Ragoussis J, Stamp GWH, Allan GJ and Trowsdale J, (1993). Frequent loss of heterozygosity on chromosome 6 in human ovarian carcinoma. Br. J. Cancer 67: 551–559.

Fujii H, Zhou W and Gabrielson E, (1996). Detection of frequent allelic loss of 6q23-25.2 in microdissected human breast cancer tissue. Genes Chromosom. Cancer 16: 35–39.

Futreal PA, Liu Q, Shattuck-Gidens D, Cochran CJ, Harshman K, Tautigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Söderkvist P, Terry L, Jhanwar S, Berchuck A, Iglehart JD, Marks

J, Ballinger DG, Barrett JC, Skolnick MH, Kamb A and Wiseman R, (1994). BRCA1 mutations in primary breast and ovarian carcinomas. Science 266: 120–122.

Gudmundsson J, Barkardottir RB, Eiriksdottir G, Baldursson T, Arason A, Egilsson V and Ingvarsson S, (1995). Loss of heterozygosity at chromosome 11 in breast cancer: association of prognostic factors with genetic alterations. Br. J. Cancer 72: 696–701.

Hainsworth PJ and Garson OM, (1990). Breast cancer cytogenetics and beyond. Aust. N.Z. J. Surg. 60: 327–336.

Hainsworth PJ, Raphael KL, Stillwell RG, Bennett RC and Garson OM, (1991). Cytogenetic features of twenty six primary breast cancers. Cancer Genet. Cytogenet. 52: 205–218.

Hainsworth PJ, Raphael KL, Stillwell RG, Bennett RC and Garson OM, (1992). Rearrangements of chromosome 1p in breast cancer correlates with poor prognostic features. Br. J. Cancer 66: 131–135.

Hill SM, Rodgers CS and Hultén MA, (1987). Cytogenetic analysis in human breast carcinoma. II Seven cases in the triploid/tetraploid range investigated using direct preparations. Cancer Genet. Cytogenet. 24: 45–62.

ISCN, (1995). Guidelines for Cancer Cytogenetics. Supplement to an International System for Human Cytogenetic Nomenclature. Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. (ed. F Mitelman).

Johansson B, Heim S, Mandahl N, Mertens F and Mitelman F, (1993). Trisomy 7 in nonneoplastic cells. Genes Chromosom. Cancer 6: 199–205.

Langston AA, Malone KE, Thompson JD, Daling JR and Ostrander EA, (1996). BRCA1 mutations in a population based sample of young women with breast cancer. N. Engl. J. Med. 334: 137–142.

Leuschner E, Meyer-Bolte K, Caselitz J, Bartnitzke S and Bullerdiek J, (1994). Fibroadenoma of the breast showing a translocation (6;14), a ring chromosome and two markers involving parts of chromosome 11. Cancer Genet Cytogenet. 76: 145–147.

Lindström E, Salford LG, Heim S, Mandahl N, Strombald S, Brun A and Mitelman F, (1991). Trisomy 7 and sex chromosomal loss need not be representative of tumor parenchyma cells in malignant glioma. Genes Chromosom. Cancer 3: 474–479.

Loupart ML, Adams S, Armour JAL, Walker R, Brammar W and Varley J, (1995). Loss of heterozygosity on the chromosome X in human breast cancer. Genes Chromosom. Cancer 13: 229–238.

Lu YJ, Xiao S, Yan YS, Fu SB, Liu Q-Z and Li D, (1993). Direct chromosome analysis of 50 primary breast carcinomas. Cancer Genet. Cytogenet. 69: 91–99.

Mackay J, Elder PA, Porteus DJ, Steel CM, Hawkins RA, Going JJ and Chetty U, (1988). Partial deletion of chromosome 11p in breast cancer correlates with the size of primary tumor and oestrogen receptor level. Br. J. Cancer 58: 710–714.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhansen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A and Skolnick MA, (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66–71.

Millikin D, Meese E, Vogelstein B, Witkowski C. and Trent T, (1991). Loss of heterozygosity for loci on the long arm of chromosome 6 in human malignant melanoma. Cancer Res. 51: 5449–5453.

Mirajver SD, Pham TM, Caduff RF, Chen M, Poy EL, Cooney KA, Weber BL, Collins FS, Johnston C and Frank TS, (1995). Somatic mutations in the BRC 1 gene in sporadic ovarian tumors. Nature Genet. 9: 439–443.

Mitchell ELD and Santibanez-Koref MF, (1990). 1p13 is the most frequently involved band in structural chromosomal rearrangements in human breast cancer. Genes Chromosom. Cancer 2: 278–289.

Mitelman F, (1994). Catalog of Chromosome Aberrations in Cancer, 5th ed., Wiley-Liss, New York.

Negrini M, Sabbioni S, Possati L, Rattan S, Corallini A, Barbanti-Broadano G and Croce C, (1994). Suppression of tumorigenicity of breast cancer cells by microcell-mediated chromosome transfer: studies on chromosomes 6 and 11. Cancer Res. 54: 1331–1336.

Pandis N, Heim S, Bardi G, Limon J, Mandahl N and Mitelman F, (1992). Improved technique for short-term culture and cytogenetic analysis of human breast cancer. Genes Chromosom. Cancer 5: 14–20.

Pandis N, Heim S, Bardi G, Idvall I, Mandahl N and Mitelman F, (1993). Chromosome analysis of 20 breast carcinomas: Cytogenetic multiclonality and karyotypic-pathologic correlations. Genes Chromosom, Cancer 6: 51–57.

Pandis N, Bardi G and Heim S, (1994). Interrelationship between methodological choices and conceptual models in solid tumor cytogenetics. Cancer Genet. Cytogenet. 76: 77–84.

Pandis N, Jin Y, Gorunova L, Petersson C, Bardi G, Idvall I, Johansson B, Ingvar C, Mandahl N, Mitelman F and Heim S, (1995). Chromosome analysis of 97 primary breast carcinomas: Identification of eight karyotypic subgroups. Genes Chromosom. Cancer 12: 173–185.

Pandis N, Idvall I, Bardi G, Jin Y, Gorunova L, Mertens F, Olsson H, Ingvar C, Beroukas K, Mitelman F and Heim S, (1996). Correlation between karyotypic pattern and clinicopathologic features in 125 breast cancer cases. Int. J. Cancer 66: 191–196.

Parkin DM, Stjernswärd J and Muir CS, (1984). Estimates of the worldwide frequency of twelve major cancers. Bull. World Health Organization 62: 163–182.

Petersen OW and van Deurs B, (1987). Preservation of defined phenotypic traits in short-term cultured human breast carcinoma derived epithelial cells. Cancer Res. 47: 856–866.

Pontén J, Holmberg L, Trichopoulos D, Kallioniemi OP, Kuale G, Wallgren A and Taylor-Papadimitriou J, (1990). Biology and natural history of breast cancer. Int. J. Cancer (Suppl.) 5: 5–21.

Rogatto SR, Rainho CA, Barbiéri-Neto J and Casartelli C, (1994). Loss of the sex chromosome in human neoplasia of the nervous system. Brazil. J. Genet. 17: 215–222.

Rohen C, Meyer-Bolte K, Bonk U, Ebel T, Staats B, Leuschner E, Gohla G, Caselitz J, Bartnitzke S and Bullerdiek J, (1995). Trisomy 8 and 18 as frequent clonal and single cell aberrations in 185 primary breast carcinomas. Cancer Genet. Cytogenet. 80: 33–39.

Scheres VMJC, (1972). Identification of two Robertsonian translocations with a Giemsa banding technique. Hum. Genet. 15: 252–256.

Sobin LH, (1981). Histological Typing of Breast Tumors, 2nd ed. World Health Organization, Geneva.

Steinarsdóttir M, Petursdóttir I, Snorradóttir S, Eyfjörd JE and Ögmundsdóttir HM, (1995). Cytogenetic studies of breast carcinomas: Different karyotypic profiles detected by direct harvesting and short-term culture. Genes Chromosom. Cancer 13: 239–248.

Thompson F, Emerson J, Dalton W, Yang JM, McGee D, Villar H, Knox S, Massey K, Weinstein R, Bhattacharyya A and Trent J, (1993). Clonal chromosome abnormalities in human breast carcinoma I. Twenty-eight cases with primary disease. Genes Chromosom. Cancer 7: 185–193.

Trent J, (1985). Cytogenetic and molecular biologic alterations in human breast cancer: a review. Breast Cancer Res. Treat. 5: 221–229.

Trent J, Yang J-M, Emerson J, McGee D, Massey K, Thompson F and Villar H, (1993). Clonal chromosome abnormalities in human breast carcinomas II. Thirty-four cases with metastatic disease. Genes Chromosom. Cancer 7: 194–203.

Vaughn JP, Davis PL, Jarboe MD, Huper G, Evans AC, Wiseman RW, Berchuck A, Iglehart JD, Futreal PA and Marks JR, (1996). BRCA1 expression is induced before DNA synthesis in both normal and tumor-derived breast cells. Cell Growth Differ. 7: 711–715.

Winqvist R, Hampton GM, Mannermaa A, Blanco G, Alavaikko M, Kiviniemi H, Taskenin PJ, Evans GA, Wright FA, Newsham I and Cavenee WK, (1995). Loss of heterozygosity for chromosome 11 in primary human breast tumors is associated with poor survival after metastasis. Cancer Res. 55: 2660–2664.

Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, Fields P, Marshall G, Narod S, Lenoir GM, Lynch H, Feunteun J, Devilee P, Cornelisse CJ, Menko FH, Daly PA, Ormiston W, McManus R, Pye C, Lewis CM, Cannon-Albright LA, Peto J, Ponder BAJ, Skolnick MH, Easton DF, Goldgar DE and Stratton MR, (1994). Localization of a breast cancer susceptibility gene, BRCA2 to chromosome 13q12-13. Science 265: 2088–2090.

Zafrani B, Gerbault-Seureau M, Mosseri V and Dutrillaux B, (1992). Cytogenetic study of breast cancer: clinicopathologic significance of homogeneously staining regions in 84 patients. Hum. Pathol. 23: 542–547.